RESEARCH ARTICLE

Cannabidiol increases gramicidin current in human embryonic kidney cells: An observational study

Mohammad-Reza Ghovanloo [1,2¤a¤b], **Samuel J. Goodchild** [1], **Peter C. Ruben** [2]*

1 Department of Cellular and Molecular Biology, Xenon Pharmaceuticals, Burnaby, BC, Canada,
2 Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada

¤a Current address: Department of Neurology, Center for Neuroscience & Regeneration Research, Yale University School of Medicine, New Haven, CT, United States of America
¤b Current address: Neuro-Rehabilitation Research Center, Veterans Affairs Connecticut Healthcare System, West Haven, CT, United States of America
* pruben@sfu.ca

Abstract

Gramicidin is a monomeric protein that is thought to non-selectively conduct cationic currents and water. Linear gramicidin is considered an antibiotic. This function is considered to be mediated by the formation of pores within the lipid membrane, thereby killing bacterial cells. The main non-psychoactive active constituent of the cannabis plant, cannabidiol (CBD), has recently gained interest, and is proposed to possess various potential therapeutic properties, including being an antibiotic. We previously determined that CBD's activity on ion channels could be, in part, mediated by altering membrane biophysical properties, including elasticity. In this study, our goal was to determine the empirical effects of CBD on gramicidin currents in human embryonic kidney (HEK) cells, seeking to infer potential direct compound-protein interactions. Our results indicate that gramicidin, when applied to the extracellular HEK cell membrane, followed by CBD perfusion, increases the gramicidin current.

Introduction

Linear gramicidins are a family of antibiotics whose function is determined by increasing the cationic permeability of the membrane [1, 2]. Increased permeability is achieved by the formation of bilayer-spanning channels via dimerization of two hemi-channels. Relative to the channels formed by other antibiotics, gramicidin (gA) is well-behaved, and forms channels that are cation selective. Gramicidin channels are also among the best-understood of these types of channels. Atomic-resolution structures have been provided, and a wealth of functional experiments have yielded important insights into gA function [3, 4]. Gramicidin channel monomers that reside in each membrane leaflet must dimerize with monomers in the other leaflet to form a continuous pore. This dimerization change is necessary and sufficient for cationic currents to be conducted through gA. The channel association/dissociation and the related energetic

cost comes from membrane deformation. The pore diameter is ~4 Å, sufficient to allow the pore to also conduct alkali metals, protons, and water [2, 5–7].

The rate of gramicidin channel dimerization is directly related to membrane stiffness or elasticity [4, 8]. This property has been the foundation of functional assays designed to determine the effects of various compounds on membrane dynamics. For example, compounds that reduce the membrane stiffness or thickness (e.g. detergents) enhance the probability of gramicidin dimerization, which in turn increases the cationic gramicidin signal [8–11].

Amphiphiles are among the compounds characterized using the gramicidin-based assays [10]. Amphiphilic compounds are a set of molecules possessing both lipophilic and hydrophilic properties. These molecules often display non-selective modulatory effects on seemingly unrelated targets, a by-product of amphiphiles modulating membrane elasticity [8–10]. Modulation is achieved when amphiphiles localize at the solution–bilayer interface, which is made possible by the compounds' polar group residing at the interface with the hydrophobic region, which then inserts into the bilayer core. Partitioning into the lipid bilayer alters membrane elasticity, and changes phase preference and membrane curvature [8–10].

One compound with amphiphilic properties is cannabidiol (CBD), the primary non-psychotropic constituent of *Cannabis sativa* [12]. CBD is a clinically and experimentally substantiated therapeutic compound with efficacy against a variety of conditions, including seizure disorders (for which CBD is FDA-approved), pain, and muscle spasms [13–19]. Furthermore, CBD has been suggested to have antibiotic properties [20, 21]. Unlike the psychotropic Δ9-tetrahydracannabinol (THC), CBD has little to no affinity for endocannabinoid receptors [22, 23]. However, many studies have shown that CBD interacts with a wide range of other targets, including a diverse array of ion channels [13, 14, 16, 17, 19, 24–26]. We previously characterized the full inhibitory effects of CBD on voltage-gated sodium channels (Nav) and deciphered the mechanism through which CBD inhibits Nav currents [17, 24, 25, 27]. We further found that an important component of this mechanism involves CBD altering membrane elasticity, which was measured using a gramicidin-based fluorescence assay (GFA) [11, 24].

GFA is based on the gramicidin permeability to Tl^+, a quencher of the water-soluble fluorophore 8-aminonaphthalene-1,3,6-trisulfonate (ANTS), encapsulated in large unilamellar vesicles (LUVs) doped with gramicidin. The rate of Tl^+ influx, measured as the rate of fluorescence quench, indicates the time-averaged number of gramicidin channels in the LUV membrane [11]. Molecules that alter the thickness and elasticity of the LUV membrane also alter the lipid bilayer contribution to the free energy of dimerization and, thus, the free energy of dimerization [28]. Our previous findings in LUVs suggested that CBD decreases gramicidin signals in that assay [24].

We sought to further characterize CBD effects on gramicidin currents using an electrophysiological HEK cell-based assay. In the present study, we investigated the interactions between gramicidin and CBD over short exposures, using voltage-clamped human embryonic kidney (HEK-293) cells in the absence and presence of gramicidin. In this purely observational study, we report that in contrast to the GFA assay, CBD increases the gramicidin current in HEK cells.

Methods

Cell culture

Suspension Human Embryonic Kidney (HEK-293) cells were used for automated patch-clamp experiments. All cells were incubated at 37 ˚C/5% CO_2. All cell culture reagents were purchased from ThermoFisher Scientific, Waltham, MA, unless otherwise noted.

Patch-clamp

Automated patch-clamp recording was performed on untransfected HEK (CLS Cat# 300192/ p777_HEK293, RRID:CVCL_0045; ATCC, Manassas, VA, USA) cells. Currents were measured in the whole-cell configuration using a Qube-384 *(Sophion A/S, Copenhagen, Denmark)* automated voltage-clamp system. Intracellular solution contained (in mM): 120 KF, 10 NaCl, 2 MgCl$_2$, 10 HEPES, adjusted to pH7.2 with CsOH. The extracellular recording solution for the high sodium experiment contained (in mM): 140 NaCl, 3 KCl, 1 MgCl$_2$, 1.5 CaCl$_2$, 10 HEPES, adjusted to pH7.4 with NaOH. For the low sodium experiment the external solution sodium concentration was lowered to 1 mM with N-methyl-D-glucamine (NMDG) as NaCl replacement. Liquid junction potentials calculated to be ~7 mV were not adjusted for. Currents were low-pass-filtered at 5 kHz and recorded at 25 kHz sampling frequency. Series resistance compensation was applied at 100%. The measurements were obtained at room temperature which corresponds to 27 ± 2 ˚C at the recording chamber. Appropriate filters for cell membrane resistance (typically >500 MΩ) and series resistance (<10 MΩ) were used. We made fresh gramicidin stock from powder (5 mg/100 μL) in DMSO (26.56 mM). Gramicidin was dissolved in 100% DMSO, and the final concentration of 26 μM. CBD was purchased from Cayman Chemicals (No. 90080) and gramicidin was obtained from Sigma-Aldrich (CAS 11029-61-1).

The Sophion Qube is an automated electrophysiology instrument that is blinded to cell selections and experimentation, and selection was performed in a randomized manner. All subsequent data filtering and analysis was performed in a non-biased manner, in which automated filters were applied to the entire dataset from a given Qube run.

Electrophysiological data analysis

The analysis of raw patch-clamp recordings was performed using the Sophion Analyzer. Graphing and additional analysis was done using the Prism GraphPad (Version 9) software.

Statistics

A one-factor analysis of variance (ANOVA) or t-test were, when appropriate, were used to compare the mean responses. *Post-hoc* tests using the Tukey Kramer adjustment compared the mean responses between channel variants across conditions. A level of significance α = 0.05 was used in all overall *post-hoc* tests, and effects with p-values less than 0.05 were considered to be statistically significant. All values are reported as means ± standard error of means (SEM) for *n* recordings/samples.

Results

CBD increases gramicidin signal in HEK cells in high extracellular sodium concentrations

Gramicidin channels preferentially conduct cationic (e.g., Na$^+$ and K$^+$) currents upon dimerization and pore formation [1, 2]. We measured cationic currents through dimerized gramicidin channels using whole-cell voltage-clamp of untransfected HEK cells in the absence and presence of 26 μM gramicidin applied to the extracellular side of the membrane. First, we measured gramicidin currents in standard high sodium [Na$^+$ = 140 mM] extracellular solution using a ramp protocol. We clamped the cell membranes at -80 mV, close to the K$^+$ equilibrium potential (E$_K^+$). Then, we hyperpolarized the cells to -120 mV and ramped the voltage to +50 mV, which is close to E$_{Na}^+$. We added the gramicidin and compound combinations after about 22 minutes, when the currents were stable, then gramicidin/compound combos were added for a 5-minute interval, and the measurements were taken at the end of the interval (**S1 Fig**).We show average gramicidin current density from the ratio of current amplitude to the
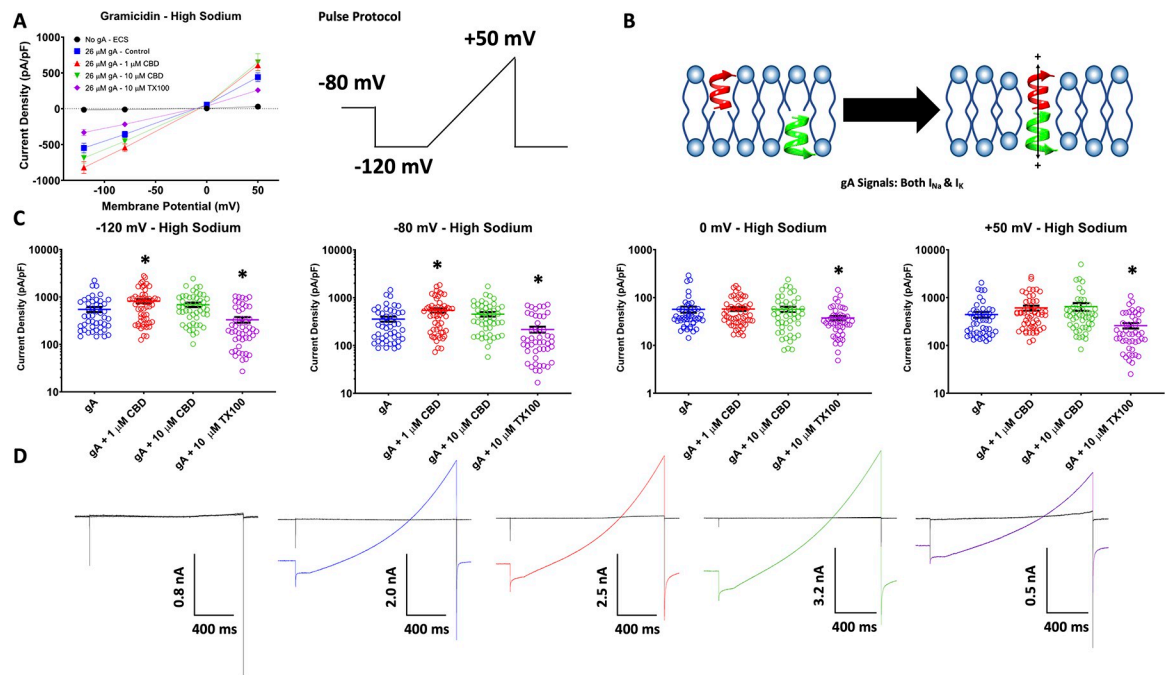
Fig 1. High sodium voltage-clamp, gramicidin (gA). (A) Shows the averaged cationic current densities of gramicidin in the presence/absence of CBD at 1 and 10 μM, and TX100 at 10 μM, on the left (in pA/pF, ECS: -120 mV = -13.6 ± 6.3, -80 mV = -10.1 ± 5.0, 0 mV = 5.2 ± 0.5, +50 mV = 30.0 ± 11.0, n = 36; gA: -120 mV = -547.8 ± 66.2, -80 mV = -357 ± 44, 0 mV = 56.5 ± 8.1, +50 mV = 441.1 ± 60.5, n = 47; 1 μM CBD: -120 mV = -820.1 ± 83.2, -80 mV = -543 ± 55.8, 0 mV = 57.7 ± 5.2, +50 mV = 607.4 ± 72.2, n = 56; 10 μM CBD: -120 mV = -687.0 ± 70.3, -80 mV = -452.8 ± 47.4, 0 mV = 57.1 ± 7.1, +50 mV = 649.2 ± 118.8, n = 49; 10 μM TX100: -120 mV = -330.8 ± 43.8 ±, -80 mV = -216.5 ± 30.4, 0 mV = 37.3 ± 4.0, +50 mV = 259.4 ± 32.8, n = 48). The ramp voltage protocol is shown on the right. (B) Shows a cartoon diagram of how gramicidin monomers are thought to dimerize and form channels. (C) Shows quantification of the data shown in (A), stars indicate statistical significance. (D) Shows the associated current traces.

cell membrane capacitance (pA/pF) at -120, -80, 0, and +50 mV (**Fig 1A–1D**). Our results indicate that, at negative potentials, gramicidin conducts inward currents and, as the membrane potential becomes more positive, the current becomes outward with the reversal potential (E_{rev}) being close to 0 mV, as would be predicted for a non-specific monovalent cationic channel. We also measured the effects of 1 μM and 10 μM CBD [17], and 10 μM Triton X100 (TX100; as positive control [9]) on gramicidin-HEK cells (**Fig 1A–1C**). TX100 is a detergent, and has been shown to change membrane elasticity and hence to increase gramicidin current amplitude in [9]. Interestingly, our findings indicate that TX100 reduced the cationic gramicidin currents across all potentials (-120 mV: p = 0.0118, -80 mV: p = 0.0123, +50 mV: p = 0.0311) (**Fig 1C**). CBD had the opposite effect to that of TX100, and slightly increased gramicidin currents at both 1 μM (-120 mV: p = 0.0152, -80 mV: p = 0.0136, +50 mV: p = 0.0368) and 10 μM (p>0.05). Interestingly, although the tendency for CBD to alter gramicidin currents was the same at both concentrations, CBD's effects were more variable at 10 μM than at 1 μM; this variability resulted in lack of statistical significance at 10 μM (**Fig 1C**). We speculate the variability at 10 μM may be due to damage to the HEK cell membrane from both gramicidin and CBD over the timescales of voltage-clamp experiments.

CBD increases gramicidin signal in HEK cells in low extracellular sodium concentrations

The presence of a gramicidin dependent current indicates ion flux across the cell membrane. Gramicidin pores are analogous to puncturing cation-selective holes through the cell
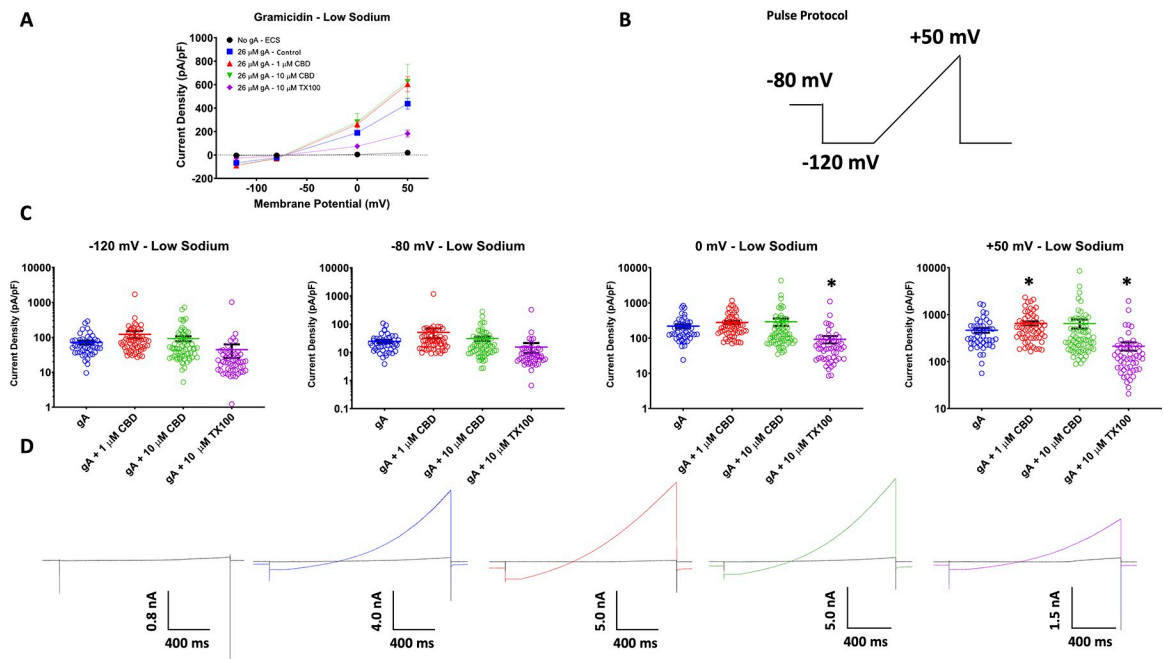
Fig 2. Low sodium voltage-clamp. Shows the averaged cationic current densities of gramicidin in the presence/absence of CBD at 1 and 10 μM, and TX100 at 10 μM (in pA/pF, ECS: -120 mV = -4.1 ± 0.9, -80 mV = -3.5 ± 0.7, 0 mV = 4.1 ± 0.4, +50 mV = 18.8 ± 1.7, n = 33; gA: -120 mV = -67.6 ± 7.7, -80 mV = -23.0 ± 2.9, 0 mV = 189.8 ± 20.6, +50 mV = 437.4 ± 45.6, n = 45; 1 μM CBD: -120 mV = -90.9 ± 9.0, -80 mV = -30.6 ± 2.9, 0 mV = 261.7 ± 28.7, +50 mV = 604.0 ± 64.8, n = 55; 10 μM CBD: -120 mV = -83.6 ± 14.1, -80 mV = -27.8 ± 5.2, 0 mV = 280.8 ± 74.4, +50 mV = 624.9 ± 149.6, n = 60; 10 μM TX100: -120 mV = -26.3 ± 3.5, -80 mV = -9.7 ± 1.3, 0 mV = 74.2 ± 11.3, +50 mV = 182.4 ± 29.0, n = 50). (B) Shows the ramp voltage protocol. (C) Shows quantification of the data shown in (A), stars indicate statistical significance. (D) Shows the associated current traces.

membrane. Gramicidin induced currents in the previous high [Na$^+$] experiment resulted in a E_{rev} close to 0 mV. This raises the possibility of a potential nonselective leak current component induced by gramicidin, but not carried by gramicidin, as a confounding variable. To ensure that we were recording gramicidin pore currents, we performed the same experiment with lower extracellular sodium [Na$^+$ = 1 mM]. This experiment resulted in the same overall trends of altered gramicidin currents densities as the high [Na$^+$] experiment, for both CBD and TX100 (Fig 2A–2D; S1 Fig). As expected, reducing [Na$^+$] lowered the gramicidin E_{rev} to ~-80 mV (close to E_K^+). These results confirm our results from the high Na$^+$ experiment and further suggest that, when both Na$^+$ and K$^+$ are present at high concentrations, gramicidin permeability is not highly selective for K$^+$ over Na$^+$ bringing the gramicidin E_{rev} to ~0 mV. Overall, these results show that CBD increases gramicidin currents during short exposures and suggests, therefore, that CBD could be altering membrane elasticity or gramicidin channel conductance directly.

Discussion

In our previous study, using a GFA, it was determined that CBD has the opposite effect to TX100 and that it decreases the rate of dimerized gramicidin channel formation, and hence a smaller gramicidin current. These findings indicate that CBD is a modifier of the bilayer physical properties at the tested concentrations of 1–30 μM [24]. In this study, by electrophysiologically measuring K$^+$ and Na$^+$ currents flowing through the gramicidin channel, the opposite result was observed. CBD increased gramicidin currents and decreased TX100 currents

suggesting an alternate mechanism of gramicidin interaction with amphiphiles like CBD or TX100 than in the GFA assay.

Although the gramicidin structure does not indicate an obvious direct binding-site for CBD [3], there is a chance of a direct CBD-gramicidin interaction taking place. Indeed, in almost every report of CBD activity on a given target, a response has been determined, including various ion channels and receptor proteins [25, 29]. Therefore, the opposite result that was observed might suggest direct CBD and gramicidin interactions that in some way increase the probability of conducting pores in these conditions.

The molecular structure of CBD is composed of two oxygen atoms on both sides of a benzene ring, with the other two ends of the ring having a hydrocarbon tail on one end, and a hydrocarbon ring on the other. These features give the CBD molecule an overall shape that is loosely reminiscent of a phospholipid molecule. Phospholipids, in turn, are molecules that specialize in separating various cellular and sub-cellular environments, a function that is dependent on their amphiphilicity. In our previous paper, molecular dynamics (MD) simulations suggested that CBD molecules tend to localize below phospholipid headgroups, but above the tail-end region [24]. Thus, CBD molecules hovered around carbons ~3–7 of aliphatic chains, as per MD and verified by NMR [24]. It is conceivable that interactions between CBD positioned in the leaflet of the HEK membrane may interact with gramicidin hemi-channels to impact pore dimerization formation in a way that offsets any membrane stiffness affects that inhibit gramicidin currents, as we saw in previous GFA studies.

In the GFA assay, gramicidin monomers are incubated for 24 hours with liposomes at 13 ˚C to reach equilibration, and then the effect of compound is investigated by measuring fluorescence quenching rates [11]. In this study, we measured conventional macroscopic cationic currents in HEK cells using standard voltage-clamp, after the cells were extracellularly perfused with gramicidin monomers over the course of minutes at 27 ˚C. Therefore, the experimental setups between the two studies are fundamentally different, and likely investigate different phenomenon pertaining to gramicidin and CBD interactions. However, CBD's effect on gramicidin currents in this study appear to be relatively weak. A potential explanation for our results could also be that because we used gramicidin in the micromolar concentrations (instead of the nanomolar used in some studies), the gramicidin monomers adopted less canonical conformations. These conformations could be the reason for why CBD's effect, which is not very strong on gramicidin are such that the currents are increased [30]. Finally, there may also be direct interactions between TX100 and gramicidin monomers in our experiments.

Our goal in this study was to describe the effects of CBD on HEK cells externally treated with gramicidin. Our results suggest that there may be a direct interaction between CBD and gramicidin but the mechanism by which this potentiates gA currents in HEK cells remains unclear. Further studies will be required for instance, using MD simulations to examine potential for direct interactions.

Supporting information

S1 Fig. Time course of experiment. (A) Shows the time course of experiment at high sodium and (B) at low sodium concentrations.
(DOCX)

Author Contributions

Conceptualization: Mohammad-Reza Ghovanloo, Samuel J. Goodchild, Peter C. Ruben.

Data curation: Mohammad-Reza Ghovanloo, Samuel J. Goodchild.

Formal analysis: Mohammad-Reza Ghovanloo, Samuel J. Goodchild.

Funding acquisition: Peter C. Ruben.

Investigation: Mohammad-Reza Ghovanloo, Samuel J. Goodchild.

Methodology: Mohammad-Reza Ghovanloo, Samuel J. Goodchild.

Project administration: Peter C. Ruben.

Resources: Samuel J. Goodchild.

Supervision: Samuel J. Goodchild, Peter C. Ruben.

Validation: Samuel J. Goodchild.

Writing – original draft: Mohammad-Reza Ghovanloo.

Writing – review & editing: Samuel J. Goodchild, Peter C. Ruben.

References

1. Harold FM, Baarda JR. Gramicidin, Valinomycin, and Cation Permeability of Streptococcus faecalis. J Bacteriol. 1967; 94: 53. https://doi.org/10.1128/jb.94.1.53-60.1967 PMID: 4961416

2. Hladky SB, Haydon DA. Discreteness of conductance change in bimolecular lipid membranes in the presence of certain antibiotics. Nature. 1970; 225: 451–453. https://doi.org/10.1038/225451a0 PMID: 5411119

3. RCSB PDB - 1NRM: Gramicidin A in Dodecyl Phosphocholine Micelles (NMR). [cited 26 Apr 2020]. Available: https://www.rcsb.org/structure/1NRM

4. Andersen OS, Koeppe RE. Bilayer Thickness and Membrane Protein Function: An Energetic Perspective. Annu Rev Biophys Biomol Struct. 2007; 36: 107–130. https://doi.org/10.1146/annurev.biophys.36.040306.132643 PMID: 17263662

5. Hladky SB, Haydon DA. Ion transfer across lipid membranes in the presence of gramicidin A. I. Studies of the unit conductance channel. BBA—Biomembr. 1972; 274: 294–312. https://doi.org/10.1016/0005-2736(72)90178-2 PMID: 5048999

6. Finkelstein A. Aqueous pores created in thin lipid membranes by the antibiotics nystatin, amphotericin B and gramicidin A. Implications for pores in plasma membranes. Drugs Transp Process B A Callingham, Ed London, UK MacMillan. 1974; 241–250. https://doi.org/10.1016/s0031-9422(00)98680-1

7. Andersen OS, Koeppe RE, Roux B. Gramicidin channels. IEEE Trans Nanobioscience. 2005; 4: 10–19. https://doi.org/10.1109/tnb.2004.842470 PMID: 15816168

8. Kapoor R, Peyear TA, Koeppe RE, Andersen OS. Antidepressants are modifiers of lipid bilayer properties. J Gen Physiol. 2019; 151: 342–356. https://doi.org/10.1085/jgp.201812263 PMID: 30796095

9. Lundbæk JA, Birn P, Hansen AJ, Søgaard R, Nielsen C, Girshman J, et al. Regulation of Sodium Channel Function by Bilayer Elasticity. J Gen Physiol. 2004; 123: 599–621. https://doi.org/10.1085/jgp.200308996 PMID: 15111647

10. Lundbæk J. CAPSAICIN REGULATES VOLTAGE-DEPENDENT SODIUM CHANNELS BY ALTERING LIPID BILAYER ELASTICITY. Mol Pharmacol. 2005; 68: 680–9. https://doi.org/10.1124/mol.105.013573 PMID: 15967874

11. Ingólfsson HI, Lea Sanford R, Kapoor R, Andersen OS. Gramicidin-based Fluorescence Assay; for Determining Small Molecules Potential for Modifying Lipid Bilayer Properties. J Vis Exp. 2010 [cited 23 Jan 2022]. https://doi.org/10.3791/2131 PMID: 20972414

12. Elsohly MA. Marijuana and the Cannabinoids. 2007. https://doi.org/10.1007/978-1-59259-947-9

13. Ross HR, Napier I, Connor M. Inhibition of recombinant human T-type calcium channels by Δ9-tetrahydrocannabinol and cannabidiol. J Biol Chem. 2008; 283: 16124–16134. https://doi.org/10.1074/jbc.M707104200 PMID: 18390906

14. De Petrocellis L, Ligresti A, Moriello AS, Allarà M, Bisogno T, Petrosino S, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol. 2011; 163: 1479–1494. https://doi.org/10.1111/j.1476-5381.2010.01166.x PMID: 21175579

15. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. N Engl J Med. 2017; 376: 2011–2020. https://doi.org/10.1056/NEJMoa1611618 PMID: 28538134

16. Kaplan JS, Stella N, Catterall WA, Westenbroek RE. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc Natl Acad Sci. 2017; 114: 11229–11234. https://doi.org/10.1073/pnas.1711351114 PMID: 28973916

17. Ghovanloo M-R, Shuart NG, Mezeyova J, Dean RA, Ruben PC, Goodchild SJ. Inhibitory effects of cannabidiol on voltage-dependent sodium currents. J Biol Chem. 2018; 293: 16546–16558. https://doi.org/10.1074/jbc.RA118.004929 PMID: 30219789

18. Iannotti FA, Pagano E, Moriello AS, Alvino FG, Sorrentino NC, D'Orsi L, et al. Effects of non-euphoric plant cannabinoids on muscle quality and performance of dystrophic mdx mice. Br J Pharmacol. 2019; 176: 1568–1584. https://doi.org/10.1111/bph.14460 PMID: 30074247

19. Fouda MA, Ghovanloo MR, Ruben PC. Cannabidiol protects against high glucose-induced oxidative stress and cytotoxicity in cardiac voltage-gated sodium channels. Br J Pharmacol. 2020; 177: 2932–2946. https://doi.org/10.1111/bph.15020 PMID: 32077098

20. van Klingeren B, ten Ham M. Antibacterial activity of Δ9-tetrahydrocannabinol and cannabidiol. Antonie Van Leeuwenhoek. 1976; 42: 9–12. https://doi.org/10.1007/BF00399444 PMID: 1085130

21. Kosgodage US, Matewele P, Awamaria B, Kraev I, Warde P, Mastroianni G, et al. Cannabidiol Is a Novel Modulator of Bacterial Membrane Vesicles. Front Cell Infect Microbiol. 2019; 9. https://doi.org/10.3389/fcimb.2019.00324 PMID: 31552202

22. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol. 1988; 34: 605–13. Available: http://www.ncbi.nlm.nih.gov/pubmed/2848184 PMID: 2848184

23. Lupica CR, Riegel AC, Hoffman AF. Marijuana and cannabinoid regulation of brain reward circuits. British Journal of Pharmacology. Wiley-Blackwell; 2004. pp. 227–234. https://doi.org/10.1038/sj.bjp.0705931

24. Ghovanloo M-R, Choudhury K, Bandaru TS, Fouda MA, Rayani K, Rusinova R, et al. Cannabidiol inhibits the skeletal muscle Nav1.4 by blocking its pore and by altering membrane elasticity. J Gen Physiol. 2021; 153. https://doi.org/10.1085/jgp.202012701 PMID: 33836525

25. Ghovanloo M-R, Ruben PC. Cannabidiol and Sodium Channel Pharmacology: General Overview, Mechanism, and Clinical Implications. Neuroscientist. SAGE PublicationsSage CA: Los Angeles, CA; 2021. p. 107385842110170. https://doi.org/10.1177/10738584211017009

26. Zhang HXB, Bean BP. Cannabidiol inhibition of murine primary nociceptors: Tight binding to slow inactivated states of Nav1.8 channels. J Neurosci. 2021; 41: 6371–6387. https://doi.org/10.1523/JNEUROSCI.3216-20.2021 PMID: 34131037

27. Sait LG, Sula A, Ghovanloo MR, Hollingworth D, Ruben PC, Wallace BA. Cannabidiol interactions with voltage-gated sodium channels. Elife. 2020; 9: 1–17. https://doi.org/10.7554/eLife.58593 PMID: 33089780

28. Rusinova R, Koeppe RE, Andersen OS. A general mechanism for drug promiscuity: Studies with amiodarone and other antiarrhythmics. J Gen Physiol. 2015; 146: 463–475. https://doi.org/10.1085/jgp.201511470 PMID: 26573624

29. Almeida DL, Devi LA. Diversity of molecular targets and signaling pathways for CBD. Pharmacol Res Perspect. 2020;8. https://doi.org/10.1002/prp2.682 PMID: 33169541

30. Andersen OS, Apell HJ, Bamberg E, Busath DD, Koeppe RE, Sigworth FJ, et al. Gramicidin channel controversy—The structure in a lipid environment. Nature Structural Biology. Nature Publishing Group; 1999. pp. 609–612. https://doi.org/10.1038/10648 PMID: 10404209